Microbiological analysis and the outcomes of periodontal treatment with or without adjunctive systemic antibiotics – a retrospective study

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Abstract

Objectives: The purpose of this retrospective study was to assess the impact of microbiological diagnostics on the outcomes of periodontal treatment with or without adjunctive use of systemic antibiotics.

Materials and methods: Patient files were screened for microbiological analysis before (T1) and after non-surgical periodontal therapy (T2). Medical history, diagnosis, clinical data and results of the microbiological analysis were extracted from the patient's file. After descriptive statistics logistic regression analysis was performed to model the presence of 90% and 50% reductions of numbers of sites with probing depths (PD) of ≥5 mm at T2 (90%-PD5 and 50%-PD5), respectively against the presence of bacterial species, clinical diagnosis, and adjunctive use of systemic antibiotics.

Results: Eighteen patients diagnosed with aggressive periodontitis (AP, 17 with adjunctive antibiotics) and 84 with chronic periodontitis (CP, 31 with adjunctive antibiotics) were included in the analysis. Logistic modeling of bacteria at T1 to 90%-PD5 failed to show any statistical significance. Using 50%-PD5, presence of all *Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola* and in particular of *T. denticola* at T1 was associated with good response to therapy. Modeling of bacterial presence to 90-%PD5 and to 50-%PD5 at T2 found an association with absence of *T. forsythia* (90-%PD5 and 50-%PD5) and of *T. denticola* and *Campylobacter rectus* (50%-PD5). Modeling bacteria at T1, antibiotic group and oral hygiene at T2 on 50%-PD5 revealed odds ratio (OR) of the adjunctive antibiotic group between 2.70 and 52.4, of the oral hygiene between 3.27 and 4.11, and of the bacteria at T1 up to 28.6 (*Porphyromonas gingivalis*, *T. forsythia*, or *T. denticola*).

Conclusion: Microbiological analysis of the most important species associated with periodontal diseases appears to support a clinically-based decision for the adjunctive use of systemic antibiotics.

Clinical relevance: The present findings appear to support the use microbiological testing to strengthen the clinical decision making process for either using or not using systemic antibiotics in conjunction with non-surgical periodontal therapy.

Key words: Microbiological analysis; periodontitis; antibiotics; response to treatment

Introduction

Periodontitis is a chronic inflammatory disease of the tooth supporting tissues associated with high counts of certain bacterial species interacting with the host' immune system [1]. Treatment protocols include removal of the biofilm from the affected teeth [2] with or without the adjunctive application of systemic antibiotics. Evidence from clinical studies indicates that the systemic use of amoxicillin combined with metronidazole significantly improves the outcomes of mechanical periodontal treatment [3, 4]. Irrespective of these results, a global problem is the development of resistance which is clearly associated with the consumption of antibiotics [5]. Nowadays, antimicrobial stewardships were implemented to prevent the rise of antimicrobial resistance, this includes fast identification of microbes and their resistance [6]. One of ten antibiotic prescriptions in human are made by dentists which might contribute to the critically important problem of bacterial resistance [7].

In periodontitis, however, biofilm consist of several hundred species [8]. Standard microbiological diagnostics of periodontitis is a nucleic-acid based one determining a few selected species with no further antibiotic resistance profile. This includes A*ggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*. *A. actinomycetemcomitans* has been associated in particular with localized aggressive periodontitis, leukotoxin, cytolethal distending toxin and lipopolysaccharide are well investigated virulence factors [9]. *P. gingivalis*, *T. forsythia* and *T. denticola* are members of the "red complex" according to the classification by Socransky, Haffajee and co-workers [10]. They occur together as late colonizers in biofilm formation, and all of them have a high proteolytic activity [11]. Meanwhile *P. gingivalis* was postulated being a key-stone pathogen in changing a symbiotic microbiota into a dysbiotic one by modulating host response [12]. Other bacterial species like *Prevotella intermedia, Parvimonas micra*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Campylobacter rectus* are additionally analyzed in microbiological test kits available on the market [13, 14].

In a previous survey it was shown that most of the European oral microbiology laboratories do not participate in external and internal quality controls [15]. Culture and PCR techniques still have methodical problems when applied in oral microbiology [16]. Sampling of plaque may influence microbiological results [17]. In 2011, a statement was published that routine analysis of subgingival plaque is not necessarily benefitting the patient [18]. Moreover, adjunctive application of antibiotics resulted in excellent clinical results regardless whether the major periodontopathogens were tested both positive or negative [19].

At the Department of Periodontology, University of Bern, a method based on DNA-strip technology (micro-IDent®*plus11* and microIDent®, Hain Lifescience, Nehren, Germany) has

been used for several years. This commercially available test is CE-labelled and has been evaluated several times [13, 20]. It is available as a five-species kit (*A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *T. denticola, P. intermedia*) and an 11-species kit (the five species plus *P. micra*, *F. nucleatum/necrophorum*, *Campylobacter rectus*, *E. corrodens*, *Eubacterium nodatum*, *Capnocytophaga* sp.). Samples for microbiological analyses are taken in a standardized manner (deepest site per quadrant) and at defined time points (before and after scaling and root planing). Additionally, the treatment protocol for periodontitis follows clearly defined guidelines. The use of systemic antibiotics is limited to the treatment of patients with Aggressive Periodontitis (AP) and severe forms of generalized Chronic Periodontitis (CP) defined as two sites with PD ≥7 mm per quadrant, and PI (O'Leary [21]) ≤0.25

However, the issue on the impact of microbiological diagnosis on the decision making process for the use or not of systemic antibiotics in conjunction with subgingival mechanical debridement is still controversially discussed in the literature.

Therefore, the aim of the present retrospective study was to evaluate the potential impact of microbiological analysis on the outcomes of periodontal therapy with and without the adjunctive use of systemic antibiotics in patients diagnosed with CP or AP.

Materials and methods

Data collection

The present study had a retrospective design including previously collected health-related patient's data were used. All available patient files were screened if 1) the patient`s agreement for the data use was provided, and 2) two microbiological tests (before and after non-surgical periodontal therapy (SRP)) were conducted.

The sample size was the available data obtained during a three-year period (2012-2014). This time frame was chosen since during this period there were no changes in the routine microbiological methods used for the analysis of subgingival plaque samples.

Inclusion criteria were the patient's agreement to use health-related data for research purposes. The minimum age was 18 years. Furthermore, according to the data file patients presented with an untreated periodontitis (no supportive periodontal therapy and no periodontal treatment within one year), the clinical diagnosis of a CP or AP and microbiological analyses at baseline (before treatment and at 3-6 months after SRP).

Additionally, all microbiological results included the first line of bacteria (*A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *T. denticola, P. intermedia*). Missing data were accepted for the other bacteria. In such situations, data were only included when they were available for both time-points.

The clinical measurements were performed at six points using the same type of manual periodontal probe (UNC15; Hu-Friedy, Chicago, IL, USA). Microbiological samples were collected from the deepest sites at baseline without removing supragingival biofilm. Following collection, the samples were pooled. The treatment protocol included oral hygiene instructions followed by supra and subgingival SRP under local anesthesia. Post-treatment biofilm control was additionally optimized by using supragingival rinses with 0.1% chlorhexidine digluconate solution twice daily according to the standard protocol of our department. In patients with AP and severe cases of CP, mechanical debridement was followed by systemic administration of amoxicillin and metronidazole (each 500 mg three times per day) for a period of 7 days.

Data from all vulnerable persons defined as age less than 18 years, pregnant women, prisoners, individuals unable to consent, were excluded. Additionally, patients receiving periodontal surgery between the two evaluation time points were excluded.

If a patient's file met the inclusion and exclusion criteria, the respective data were captured. This information included patient-related data (gender, age in years, systemic diseases (yes/no), and smoking habits), clinical data (diagnosis, number of teeth, number of sites with Probing Depths (PD) ≥5 mm, number of sites with Bleeding on Probing (BOP) as well as oral hygiene status expressed as plaque index according to O'Leary [21]. The results of the microbiological analysis were classified as follows for each bacterial species: 0 = no detection, 1 = weak load, 2 = moderate load, and 3 = heavy load).

The study protocol was submitted to and approved by the ethical commission of the Canton of Bern, Switzerland (Basec # 2016-00930).

Data analysis

Data processing calculated the per cent of reduction of sites with PD≥5 mm at T2 in comparison with T1. In addition, categorical data for bacterial species were dichotomized and the sum of positive results was calculated per patient for *P. gingivalis*, *T. forsythia* and *T. denticola* (and *A. actinomycetemcomitans*), respectively.

The primary outcome variable was the presence of *P. gingivalis*, *T. forsythia* and *T. denticola* (sum) at T1 in patients demonstrating a 90% reduction in the number of sites with PD ≥5 mm at T2 (90%-PD5). Secondary endpoints were the presence and categories of the other analyzed bacteria and with 50% reduction of numbers of sites with PD ≥5 mm at T2 (50%-PD5). Additionally, the influence of the adjunctive use of antibiotics, the clinical diagnosis (e.g. AP or CP), the level of oral hygiene and of smoking habits on the clinical and microbiological outcomes were evaluated.

Following descriptive statistics on all variables, a logistic regression analysis was performed to model the presence of 90%-PD5 against the sum of positive results of *P. gingivalis*, *T. forsythia* and *T. denticola*. Furthermore, the influence of the different variables was investigated with multiple regression analysis and general linear models for the analysis of variance and covariance. Secondary outcomes were compared between the groups. Statistical analysis was performed using the SAS POUZ LOGISTIC and GLM [22]. The level of significance was set to p=0.05.

Results

Study population and clinical variables

A total of 102 patients (49 male, 53 female; mean age 46.1 years, range 19 – 81 years) fulfilled the inclusion criteria. The patients were diagnosed and treated by nine clinicians (8 – 20 patients per clinician). Eighteen patients had been diagnosed with AP and 84 with CP, respectively. The clinical data including PD at sampling sites at T1 and T2 are summarized in Table 1.

Microbiological outcomes

Dichotomized data revealed differences between CP and AP at T1 with a higher prevalence of *A. actinomycetemcomitans*, *E. nodatum* and a lower prevalence of *F. nucleatum*/*necrophorum* in AP. Except for *A. actinomycetemcomitans, F. nucleatum* and *Capnocytophaga* sp. the presence of all other bacteria was reduced at T2 in comparison with T1 both in AP and CP patients, respectively. In patients with AP, *A. actinomycetemcomitans* was detected less frequently by tendency following non-surgical therapy.

Analyzing if any of the bacterial species *P. gingivalis*, *T. forsythia*, *T. denticola* (and *A. actinomycetemcomitans*) was present in AP and CP patients revealed statistically significant

less positive results following non-surgical therapy in comparison to baseline. At T2, more positive results were found in CP than in AP patients (Table 2).

Bacteria and response to treatment

In patients with 90%-PD5, the presence of *P. gingivalis*, *T. forsythia*, *T. denticola* (sum) at T1 did not present any statistically significant association (primary outcome variable). There was also no detectable association with 90%-PD5 when dichotomized data at T1 were employed. However, when using 50%-PD5, higher presence of *T. denticola* at T1 was associated with good response to therapy. Moreover, certain categories of bacterial counts (*T. forsythia* (3), *T. denticola* (2), *C. rectus* (2) as well as the combined presence of *P. gingivalis*, *T. forsythia* and *T. denticola*) were associated with good response to treatment (odds ratio (OR) between 4.67 and 13.50).

At T2, 90%-PD5 was statistically significantly associated with lower presence of *T. forsythia* and *E. corrodens*. In 50%-PD5, the presence of *T. forsythia*, *T. denticola*, *C. rectus* and the sum of positive results of *P. gingivalis*, *T. forsythia* and *T. denticola* together with certain single categories of analyzed bacteria, were negatively associated with the clinical outcomes (Table 2).

Furthermore, we modeled the disease severity (defined by ≤20 sites with PD ≥5 mm, >20 sites with PD ≥5) on 90%-PD5 and 50%-PD5. Any influence on treatment outcome by disease severity group was not seen (data not shown).

Treatment outcome in association with the presence of bacteria, antibiotic treatment related to clinical diagnosis of periodontitis

Seventeen out of the 18 AP patients received adjunctive antibiotics to non-surgical therapy antibiotics (AP-AB). The patient diagnosed with AP and treated without antibiotics was excluded from that analysis. Of the 84 CP patients, systemic antibiotics were administered to 31 patients (CP-AB), whereas 53 patients were treated with non-surgical therapy alone (CP-noAB).

Using a general logistic model at T1, the clinical results revealed a difference between the subgroups. More specifically, the subgroup CP-AB presented with more sites with PD ≥5 mm than the subgroup CP-noAB. All clinical parameters were improved in all three subgroups

after non-surgical therapy. At T2 the PI revealed statistically significant differences. In both groups treated with adjunctive antibiotics (AP-AB, CP-AB), the plaque level was statistically significantly lower than in CP-noAB (Table 4).

The sum of *P. gingivalis*, *T. forsythia, T. denticola* and *A. actinomycetemcomitans* decreased in each subgroup statistically significantly after non-surgical therapy. At T1 there was no difference between the subgroups, at T2 the value was higher in CP-noAB compared with AP-AB and CP-AB (Fig. 2).

As the PI was different at T2 between the subgroups, logistic modeling included presence of bacteria at T1, antibiotic subgroup and PI at T2. The variable plaque score was dichotomized by using a cut-off of 20%. Logistic modeling to 90%-PD5 did not show any statistical significance. Using 50%-PD5 as a criterion, all models including first-line bacteria were statistically significant positive with $p<0.01$. In these models the impact (OR) of the adjunctive antibiotic group was between 2.70 and 52.4, the OR of PI was between 3.27 and 4.11 and the OR of bacteria at T1 ranged between 1.72 and 28.6 (any of *P. gingivalis*, *T. forsythia*, *T. denticola*; Table 5).

Treatment outcome in association with presence of bacteria and smoking

Among the patients included in the analysis 57 (56%) never smoked, from the 39 (38%) active smokers at T1 four patients stopped smoking during non-surgical periodontal therapy. Sixteen (61%) of the patients never smoked in the group demonstrating 90%-PD5, and 41 (54%) in the group without 90%-PD5. There were no statistically significant differences in terms of bacterial presence at T1 and T2 between the smoking groups. When using GLM to model the impact of smoking, bacteria and antibiotics on 90%-PD5 and 50%-PD5 no statistically significant influence of smoking on response to treatment was found (p between 0.233 and 0.560 for 90%-PD5 and between 0.342 and 0.940 for 50%-PD5).

Discussion

The present retrospective analysis has attempted to shed light on the clinically extremely important, but still controversially discussed, issue namely, the impact of microbiological diagnosis on the outcomes of periodontal treatment with or without adjunctive use of systemic antibiotics. The results appear to suggest that patients harboring before therapy *P.*

gingivalis, *T. denticola* and *T. forsythia* may benefit mostly from an adjunctive use of systemic antibiotics in conjunction with non-surgical therapy. Despite obvious limitations due to the retrospective nature of the study, the present data appear to indicate a potential benefit of performing microbiological diagnosis when considering the use of systemic antibiotics in conjunction with nonsurgical periodontal therapy.

In our laboratory a nucleic-acid based strip-technology (micro-IDent®*plus11 and* microIDent®, Hain Lifescience, Nehren, Germany) is used for routinely sampled subgingival biofilm, procedure in laboratory is according to the manufacturer's instruction. The test is able to identify 11 or five periodontopathogenic bacterial species. First, DNA is extracted by the Chelex method [23]. Thereafter, two (11 species) or one (five species) PCR runs are performed before subsequent reverse hybridization and analysis. In our laboratory the resulting bands for positive reactions are compared visually and categorized independently by two experienced persons. Sensitivity and specificity of the test kits was shown in several studies [13, 14]. Cut-off of the test confirmed for single species in laboratory is 10^3 for *A. actinomycetemcomitans* and 10^4 for the other species. Our own evaluation revealed an increase by about one log10 when several bacterial species to be detected in a sample are present. In most of the final analyses only dichotomized data of bacteria were included since no more differentiating results were obtained when using categories. The used cut-off of 10^4 was tested to be clinical relevant for *P. gingivalis* [24]. Consequently, it may be sufficient and probably helpful also for the clinicians' understanding to provide only qualitative results from laboratory when test system with cut-off is used.

Meanwhile, new technologies allow the identification of species present in a complex microbiome. These investigations allow a better understanding of a complex ecosystem but are difficult to interpret by the clinician. Therefore, medical microbiology still focuses primarily on selected bacteria when analyzing an ecosystem (for example in cystic fibrosis, only certain bacterial species are used to identify the infection [25]). Despite the fact that the subgingival biofilm consists of about 1'000 species [26], only 11 species were assessed in that study. However, statistically significant results were mainly obtained for the three members of the so-called "red complex" underlining once more the importance of *P. gingivalis, T. forsythia* and *T. denticola*. These bacterial species are those most associated with periodontal disease [27].

According to the standard protocol used at the Department of Periodontology, University of Bern, the four deepest sites (preferably one per quadrant) are chosen. After air-drying and isolation by cotton rolls, each an endodontic paper point (ISO 50) is inserted into the selected site until resistance is felt for 20s. The supragingival biofilm is not removed before sampling.

The samples from the four different sites are pooled in the transport vials and immediately transferred to laboratory where they are stored at -20°C until analysis, which is regularly performed within one week. This approach is based on the findings which have shown that the microbiota is not very different in pockets with similar probing depths, irrespective of the region [28]. Detection frequency of periodontopathogens is higher when using pooled samples [17] and when supragingival plaque is not removed [29].

Comparing AP and CP, more positive results of *A. actinomycetemcomitans* and *E. nodatum* were found in AP patients. Regarding *A. actinomycetemcomitans*, this finding confirms those of a recent study where about 70% of positive samples were detected in patients diagnosed with localized AP [30]. In our study, half of the AP patients have been tested positively for *A. actinomycetemcomitans*. When interpreting these findings it has to be kept in mind that the clinical diagnosis of AP is often difficult [31]. Genotyping of *A. actinomycetemcomitans* for genotype b and c was not included in our statistical analysis due to the low numbers of samples being tested positively for that species. Genotype b was detected in 6 patients, whereas genotype c was found in 5 patients. Only once deletion within leukotoxin gene was present in a patient originating from North Africa. This is in line with the high prevalence of that clone in North Africa [32]. Other bacteria might contribute to a microbial dysbiosis triggering a rapid destruction of periodontal tissue. A recent study found *A. actinomycetemcomitans* in five of 19 biofilm samples of Sudanese AP patients whereas all samples of 15 periodontally healthy controls were negative [33]. Here, *E. nodatum* was also more prevalent in AP than in controls [33] which may support a potential role of that species in AP.

PD ≥5 mm represents a risk factor for further attachment and tooth loss [34]. Clinical outcome was defined as per cent of reduction of sites with PD ≥5 mm after non-surgical therapy. We did not detect an influence of the initial numbers of sites of PD ≥5 mm in our statistical evaluation. Two different levels were used to define high response to treatment, 90% per cent and 50% of reduction of sites with PD ≥5 mm at T2 in comparison with T1. In nearly all analyses (incl. the primary outcome presence of *P. gingivalis*, *T. forsythia*, *T. denticola* (sum) at T1), no influence of microbiological result on 90%-PD5 was observed. However, using 50%-PD5 revealed several interesting results. Presence of all *P. gingivalis, T. forsythia and T. denticola* and in particular of *T. denticola* at T1 und opposite, absence of any *P. gingivalis, T. forsythia, T. denticola* and in addition of *C. rectus* at T2 was associated with high response to therapy. The baseline findings of the present study are in line with those of a recent report, where higher proportions of *Porphyromonas* sp., *Treponema* sp. at baseline predicted a better treatment outcome [35]. Since the etiology of periodontitis is

linked with the prevalence of these bacterial species at high numbers, an effective elimination (below the detection level) improves obviously the clinical outcomes.

Mechanical non-surgical therapy remains the gold standard in the treatment of periodontitis as it removes or destroys the subgingival biofilm [36]. Models confirmed the influence of selected bacteria (in particular of *P. gingivalis, T. forsythia and T. denticola*) on treatment outcome also when adjunctive antibiotics were applied. The protocol used in our Department considers the administration of amoxicillin and metronidazole only given in conjunction with non-surgical periodontal therapy. This combination has been repeatedly demonstrated to be beneficial for the treatment of AP [37]. Furthermore, recent systematic reviews have also demonstrated a profound effect of this combination upon clinical outcomes in CP patients [38]. In the present study, about half of the analyzed patients received adjunctive amoxicillin and metronidazole. However, it should be kept in mind that in the routine clinical setting, this number is probably lower since sampling for microbiological diagnostics is often linked with adjunctive antibiotic therapy. As shown in the baseline data, mean PD was higher in CP patients who received adjunctive antibiotics. However, the discussion about the role of adjunctive antibiotics in the treatment of CP is still controversial. While some authors recommend the routine use of adjunctive antibiotics [39, 40] others recommend a very restricted use [41]. On the other hand, the present findings are in line with those of a recent report on the changes in the subgingival microbiome up to one year post-treatment of patients treated with non-surgical periodontal therapy with or without amoxicillin and metronidazole pointing to the predictive value of specific subgingival bacterial profiles for the decision to prescribe antibiotics in the treatment of periodontitis [35].

Our logistic analysis confirmed an impact of adjunctive antibiotics on the treatment response. However, antibiotics have side effects, *e.g.*, 1-2% of individuals have allergy to penicillins [42], metronidazole was discussed having ototoxicity [43] and combined with penicillin it may cause nausea and diarrhea [44]. Moreover, the global problem of development of resistance associated with the consumption of antibiotics [45] demands a well indicated and very restricted use of these drugs [46]. *E.g.*, World Health Organization is celebrating the “World Antibiotic Awareness Week” once per year, among others dentists are asked to handle antibiotics with care [47]. After non-surgical periodontal therapy, the PI was lower in the groups that received systemic antibiotics. Including this variable in logistic models underlines the impact of good oral hygiene on response to therapy. Since antibiotics do not destroy biofilms [48, 49], a potential psychological effect leading to improved oral hygiene measures, has also to be suggested in the patients receiving these highly potent drugs.

Limitations of the study are those of a retrospective study in general. Data were recorded as they appeared in the patient`s files and thus, potential bias in data recording cannot be completely excluded. Although guidelines for diagnostics and periodontal treatment exist, an influence by the individual clinician cannot be completely ruled out. The interval between the two time-points was in a range of 3 to 6 months. Moreover, non-recorded (*e.g.*, due to not reporting by the patient) antibiotic intake for medical reasons might have also influenced the results. Furthermore, with a total number of 102 patients analyzed in this study, the final sample size was not very high. Consequently, potential differences between the groups may fail to reveal statistical significances.

In summary, the present findings suggest that microbiological detection of the most important species associated with periodontal disease (*P. gingivalis*, *T. forsythia*, *T. denticola*, *A. actinomycetemcomitans*) appears to be sufficient to indicate microbial dysbiosis, and may help the clinician in the decision making process of using or not systemic antibiotics during non-surgical periodontal therapy.

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Compliance with ethical standards

Conflict of Interest: The authors declare that they have no conflict of interest.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: In this retrospective study, an informed consent was not obtained from all individual participants. However, agreement for further data use was marked in all patients' files included for analysis.

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Table 1
Epidemiological and clinical data

Variable	T1: mean±SD	T2: mean±SD
Total (n)	102	102
Gender: male/female	49/53	
Age: mean (range)	46.1 (19-81)	
Clinical diagnosis: AP/CP	18/84	
PD≥5 mm (n/patient)	39.61±26.27	12.78±13.77
BoP (%)	48.39±22.72	19.67±14.36
O'Leary (%)	48.53±24.34	22.02±15.84
PD (mean of the four sampling sites/patient)	7.29±1.63	5.08±1.28
Diff PD ≥5 mm (n)		26.82±22.52
Diff PD ≥5 mm (%)		65.83±27.24
Smoking: Non-smokers / smokers / former smokers (n)	57/39/6	57/35/10
90%-PD5 (n (%))*		26 (25.5)
50%-PD5 (n (%))*		76 (74.5)

***90% or 50% reduction in the number of sites with PD ≥5 mm at T2

Table 2
Presence of selected bacterial species (qualitative data) at baseline (T1) and after non-surgical therapy (T2) related to the clinical diagnosis of aggressive periodontitis (AP) and chronic periodontitis (CP)

Bacterium		AP (n=18) n (%)	CP (n=84) n (%)	P AP vs. CP
A. actinomycetemcomitans				
	T1	7 (38.9)	11 (13.1)	0.016*
	T2	2 (11.1)	13 (15.5)	1.000
	P	0.096	0.593	
P. gingivalis				
	T1	14 (77.8)	48 (57.1)	0.119
	T2	3 (16.7)	25 (29.8)	0.385
	P	<0.001**	<0.001**	
T. forsythia				
	T1	17 (94.4)	77 (91.7)	1.000
	T2	4 (22.2)	46 (54.8)	0.018*
	P	<0.001**	<0.001**	
T. denticola				
	T1	16 (88.9)	72 (85.7)	1.000
	T2	5 (27.8)	36 (42.9)	0.295
	P	<0.001**	<0.001**	
P. intermedia				
	T1	9 (50.0)	41 (48.8)	1.000
	T2	2 (11.1)	15 (17.9)	0.730
	P	0.020*	<0.001**	
P. micra				
	T1	8 (53.3)	43 (67.2)	0.374
	T2	2 (13.3)	21 (32.8)	0.208
	P	0.034*	<0.001**	
F. nucleatum/necrophorum[1]				
	T1	13 (86.7)	64 (100.0)	0.034*
	T2	10 (66.7)	55 (85.9)	0.126
	P	0.257	0.003**	
C. rectus [1]				
	T1	12 (80.0)	57 (89.1)	0.391
	T2	5 (33.3)	27 (42.2)	0.575
	P	0.008**	<0.001**	
E. nodatum[1]				
	T1	9 (60.0)	17 (26.6)	0.029*
	T2	1 (6.7)	6 (9.4)	1.000
	P	0.005**	0.005**	
E. corrodens[1]				
	T1	10 (66.7)	47 (73.4)	0.750
	T2	4 (26.7)	33 (51.6)	0.094
	P	0.014*	0.003**	
***Capnoc.* sp.** [1]				
	T1	11 (73.3)	34 (53.1)	0.246
	T2	9 (60.0)	29 (45.3)	0.393
	P	0.480	0.336	
***Pg, Tf, Td* (any)**				
	T1	17 (94.4)	81 (96.4)	0.546
	T2	5 (27.8)	53 (61.9)	0.010
	P	<0.001**	<0.001**	
***Pg, Tf, Td, Aa* (any)**				

	T1	17 (94.4)	81 (96.4)	0.546
	T2	6 (33.3)	54 (64.3)	0.019
	P	<0.001**	<0.001**	

[1]The second line species were analyzed at two time-points only in 15 patients with AP and 64 patients with CP.

Intra-group differences were determined by using Mc Nemar's test, inter-group differences by using Fisher exact test.

* significant difference p<0.05

** significant difference p<0.01

Table 3
Association of presence of bacteria at T1 and T2 as well as of categories (3 heavy load, 2 moderate load, 1 weak load, 0 no detection; for single categories only significant results vs. 0 are mentioned) with success of therapy (90% and 50% of reduction of sites with PD ≥5mm)

Bacteria	**Time-point**	**90% of reduction of sites with PD ≥5mm**			**50% of reduction of sites with PD ≥5mm**		
		0;1		**categories**	**0;1**		**categories**
		P; OR [CI]	**all (P)**	**vs. 0 Load: P; OR [CI]**	**P; OR [CI]**	**all (P)**	**vs. 0 Load: P; OR [CI]**
A. actin.	T1	n.s.	n.s.		n.s.	n.s.	
	T2	n.s.	n.s.		n.s.	n.s.	
P. gingivalis	T1	n.s.	n.s.		n.s.	n.s.	
	T2	n.s.	n.s.		n.s.	n.s.	
T. forsythia	T1	n.s.	n.s.		n.s.	n.s.	3: 0.039; 6.25 [1.10;35.68]
	T2	0.019; 0.31 [0.12; 0.82]	n.s.	2: 0.039; 0.28 [0.09;0.92]	<0.001; 0.15 [0.05;0.43]	0.004	1: 0.001; 0.09 [0.02;0.38] 2: 0.003; 0.17 [0.05;0.54]
T. denticola	T1	n.s.	n.s.		0.030; 3.63 [1.13; 11.63]	n.s.	2: 0.013; 4.67 [1.38;15.79]
	T2	n.s.	n.s.	2: 0.048; 0.27 [0.07;0.99]	0.000; 0.24 [0.09; 0.63]	0.012	2: 0.013; 0.27 [0.10;0.76]
P. intermedia	T1	n.s.	n.s.		n.s.	n.s.	
	T2	n.s.	n.s.		n.s.	n.s.	
P. micra	T1	n.s.	n.s.		n.s.	n.s.	

	T2	n.s.	n.s.	n.s.	n.s.	
F. nucl./necr.	T1	n.s.	n.s.	n.s.	n.s.	
	T2	n.s.	n.s.	n.s.	n.s.	
C. rectus	T1	n.s.	n.s.	n.s.	n.s.	2: 0.027; 8.67 [1.28;58.85]
	T2	n.s.	n.s.	0.007; 0.18 [0.05; 0.63]	0.030	1: 0.026; 0.16 [0.03;0.81] 2: 0.006; 0.11 [0.02;0.54]
E. nodatum	T1	n.s.	n.s.	n.s.	n.s.	
	T2	n.s.	n.s.	n.s.	n.s.	
E. corrodens	T1	n.s.	n.s.	n.s.	n.s.	
	T2	0.028; 0.28 [0.09; 0.87]	n.s.	n.s.	n.s.	
***Capnoc.* sp.**	T1	n.s.	n.s.	n.s.	n.s.	
	T2	n.s.	n.s.	n.s.	n.s.	
***P. g.*, *T.f., T.d.*[1]**	T1	n.s.	n.s.	n.s.	n.s.	3: 0.031; 13.50 [1.27;143.64]
	T2	n.s.	n.s.	0.002; 0.155 [0.05; 0.49]	<0.001	2: <0.001; 0.07 [0.02;0.26] 3: 0.039; 0.23 [0.06;0.93]

***A. a., P. g., T.f., T.d.*[1]**	T1	n.s.	n.s.	n.s.	n.s.	
	T2	n.s.	n.s.	0.004; 0.18 [0.06; 0.58]	0.008	2: 0.003; 0.03 [0.02;0.50] 3: 0.001; 0.11 [0.03;0.42]

[1]presence (0;1): any; categories: sum of presence

Table 4

Clinical variables related to clinical diagnosis of aggressive periodontitis (AP) and chronic periodontitis (CP) and with adjunctive antibiotic treatment (AB) and without antibiotic (noAB) at baseline (T1) and after non-surgical therapy (T2)

	AP-AB (n=17)		CP-AB (n=31)		CP-noAB (n=53)		P (GLM)		P single statistically significant results	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
PD≥5 mm (n: mean ± SD)	43.6±32.7	10.2±11.2	48.7±29.7	11.8±13.4	32.7±19.9	13.6±14.4	0.019	0.635	CP-AB vs. CP-NoAB: 0.007	
P (Student) T2 vs. T1		<0.001		<0.001		<0.001				
BOP (%: mean ± SD)	48.0±24.1	17.7±16.5	48.5±23.3	16.9±16.1	48.0±22.4	21.4±11.9	0.995	0.321		
P (Student) T2 vs. T1		<0.001		<0.001		<0.001				
O'Leary (%: mean ± SD)	40.7±28.0	14.3±9.02	44.5±24.0	16.5±11.1	52.6±22.2	27.4±11.8	0.125	<0.001		AP-AB vs. CP-noAB: 0.002 CP-AB vs. CP-NoAB: 0.002
P (Student) T2 vs. T1		<0.001		<0.001		<0.001				

Table 5

Logistic modelling of presence of bacteria at T1, O'Leary plaque score <20% at T2 and antibiotic group (aggressive periodontitis with adjunctive antibiotics (AP-AB), chronic periodontitis with adjunctive antibiotic treatment (CP-AB) and without antibiotic (CP-noAB)

Species	**90%-PD5**	**50%-PD5**	**OR of statistically significant results 50%PD5**			
	P	**P**	**bacteria 1;0 at T1**	**O'Leary 0;1 at T2**	**AP-AB vs. CP-noAB**	**CP-AB vs. CP-noAB**
A. actin.	n.s.	0.003	3.29 [0.57; 18.8]	4.11 [1.36; 12.5]	2.70 [0.50; 14.6]	14.9 [1.83; 122]
P. gingivalis	n.s.	0.003	2.02 [0.70; 5.89]	3.27 [1.10; 9.72]	3.25 [0.63; 16.8]	18.5 [2.23; 153]
T. forsythia	n.s.	0.008	15.9 [1.31; 192]	3.48 [1.13; 10.8]	4.40 [0.78; 25.0]	52.4 [2.96; 926]
T. denticola	n.s.	0.002	4.93 [1.16; 21.0]	3.39 [1.12; 10.3]	4.15 [0.75; 23.0]	20.3 [2.29; 179]
P. intermedia	n.s.	0.004	1.72 [0.60; 4.95]	3.60 [1.21; 10.6]	3.76 [0.73; 19.3]	18.0 [2.18; 149]
P. micra	n.s.	n.s.				
F. nucl./necr.	n.s.	0.047	8.12 [0.23; 288]	3.30 [0.90; 12.2]	3.25 [0.35; 30.1]	8.04 [0.93; 69.5]
C. rectus	n.s.	0.036	5.58 [1.01; 29.5]	4.57 [1.14; 18.3]	2.41 [0.37; 15.6]	8.49 [0.91; 79.3]
E. nodatum	n.s.	n.s.				
E. corrodens	n.s.	n.s.				
***Capnoc.* sp.**	n.s.	n.s.				
P. g.*, *T.f., T.d.	n.s.	0.004	28.6 [1.72; 477]	3.82 [1.23; 11.9]	4.87 [0.80; 29.6]	28.4 [2.18; 369]
A. a., P. g.*, *T.f., T.d.	n.s.	0.004	28.6 [1.72; 477]	3.82 [1.23; 11.9]	4.87 [0.80; 29.6]	28.4 [2.18; 369]

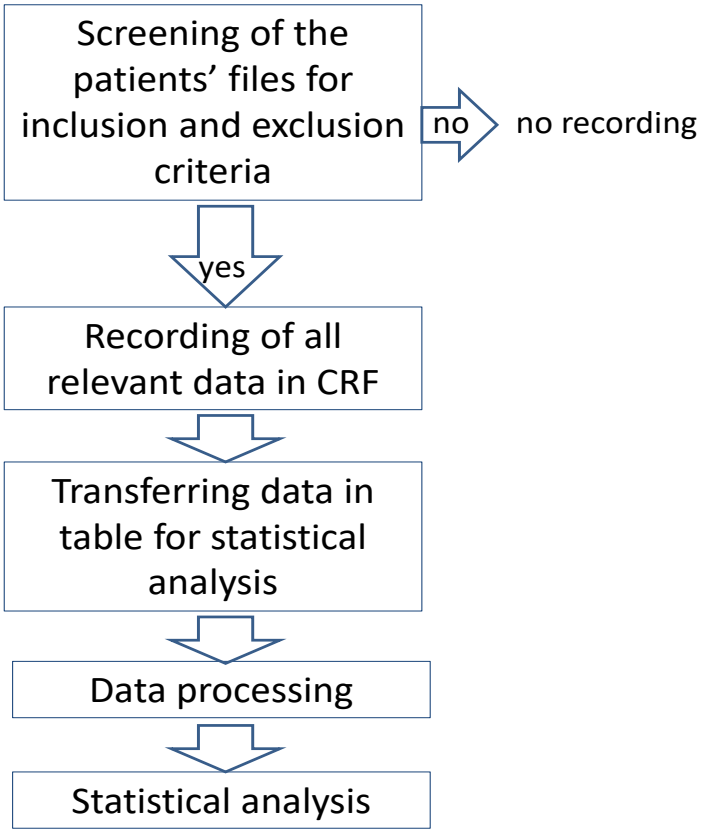

Fig. 1
Flow chart of the data assessment and analyses

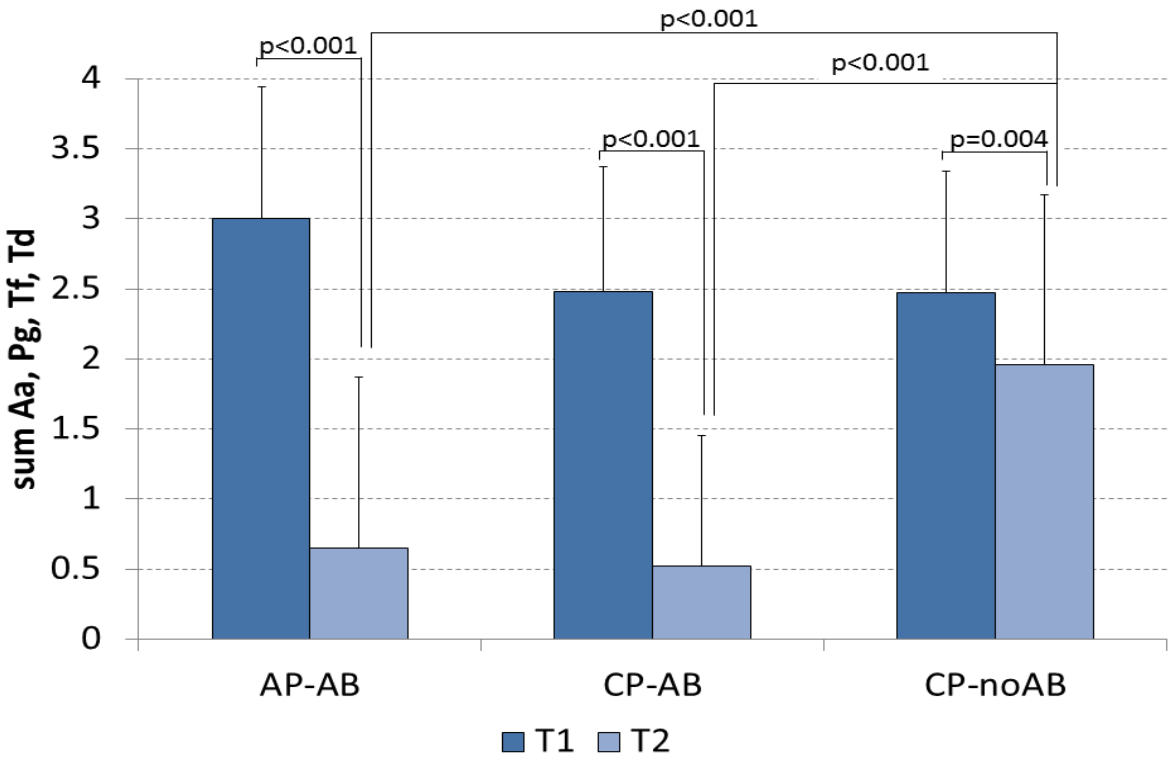

Fig. 2
Sum of positive results / sample of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* (mean and SD) in subgingival plaque obtained in aggressive periodontitis with adjunctive antibiotics (AP-AB), chronic periodontitis with adjunctive antibiotics (CP-AB) and chronic periodontitis without adjunctive antibiotics (CP-noAB) at baseline (T1) and after non-surgical therapy
(Wilcoxon two-sample and Wilcoxon matched-pairs tests)